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# A SEARCH FOR AMINO ACIDS IN APOLLO 11 AND 12 LUNAR FINES*

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SUMMARY

Lunar fines from the Apollo 11 and 12 missions have been analyzed for amino acids and a wide range of other derivatizable organic compounds by a highly sensitive gas–liquid chromatographic method[6]. A minimum of 3 to 5 p.p.b. of each amino acid would have been detected, but there were no indications of the presence of free amino acids in the samples.

The N-trifluoroacetyl *n*-butyl ester/gas–liquid chromatographic method has been shown to give recoveries greater than 80% when samples containing 5 ng of each of seventeen amino acids were taken through the total derivatization and chromatography, thus showing that losses due to adsorptive and substrate-derivative interactions were minimal. The method was also shown capable of detecting other organic classes as higher alcohols, amines, fatty acids, and dicarboxylic acids.

The unhydrolyzed water reflux extract (17 h) of the Apollo 11 fines showed that the concentration of each of the free common amino acids were less than 4 ng/g, however, on hydrolysis of the water extract with 6 *N* HCl, a large peak was eluted on the glycine–serine region of the chromatogram, and two other peaks at 153° (80 ng/g) and 178° (40 ng/g). The large peak could be contamination as a smaller peak was observed at the same retention temperature in the parallel blank; or these peaks could be due to organic precursors which are hydrolyzed as reported by Fox[10].

The Apollo 12 unhydrolyzed water reflux extract (13 h) was also investigated, and none of the free common amino acids were detected above background (*ca.*

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4 ng/g each). However, several unidentified chromatographic peaks were observed which were not present in the corresponding procedural blank. These substances which appear to be derivatizable, are significantly above background level, and could either be indigenous to the Apollo 12 sample, the result of the unusual chromatographic effect due to unknown substances in the samples reported by us earlier[8], or contamination. An analysis of the hydrolyzed Apollo 12 water extract was not made.

Analyses were made on "clean fingerprints" which resulted in the detection of 1000 ng total of amino acids per fingerprint.

An injection port solvent-venting system has been developed which has greatly simplified the gas–liquid chromatographic analysis of nanogram amounts of amino acids[6]. This device allows the injection of the total derivatized samples (up to 100 $\mu$l) on a standard packed analytical column and prevents the solvent and derivatizing reagents from traversing the column and entering the detector. This instrumental modification will be of great value in analyses on future Apollo samples.

A series of collaborative experiments have been designed by the participating organic principal and co-investigators (PONNAMPERUMA, KVENVOLDEN, GEHRKE, FOX, HARE, HARADA; NAGY, HAMILTON; ORO) to critically evaluate and optimize the gas–liquid and classical ion-exchange methods used prior to analysis of the Apollo 14 samples. These experiments will include: (a) assessment of sensitivity of methods on a series of standard amino acids solutions at the 5, 20, and 100 ng levels by each group, and, (b) analyses for amino acids in unhydrolyzed and hydrolyzed water extracts of a single homogeneous sample of Onverwacht chert to evaluate hydrolysis, adsorption and analyses of the 20 to 60 p.p.b. levels in chert.

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## INTRODUCTION

The examination of extraterrestrial materials for indigenous amino acids presents possibilities for gaining information on the origin and nature of early life forms, and whether or not amino acids might be synthesized in the primitive environments of interstellar space. The lunar samples returned by Apollo flights 11 and 12 have been studied extensively in search for biologically important compounds[1–3].

The quantity of organic material present in Apollo 11 lunar samples has been reported to be quite small[1], thus requiring highly sensitive analytical methods for the detection and identification of amino acids which may be present. Further, diverse sources of possible sample contamination[4] add to the complexity of obtaining an unequivocal analysis for amino acids which might be indigenous to the lunar samples, as a single fingerprint results in 500 ng or more of amino acids.

To minimize contamination, all analyses were carried out in an ultraclean laboratory at Ames, designed specifically for lunar sample work. A highly sensitive gas–liquid chromatographic (GLC) technique developed by GEHRKE AND ZUMWALT was used[5–7] to determine whether detectible amounts of amino acids were present or absent in Apollo 11 and 12 lunar fines. With this method, the N-trifluoroacetyl (N-TFA) *n*-butyl ester derivatives are formed by esterification of the amino acids with *n*-butanol·3 *N* HCl followed by acylation with trifluoroacetic anhydride. This investigation also centered on other classes of organic compounds in addition to amino

acids that could be detected under the derivatization and chromatographic conditions used. Included were an alcohol, an amine, a fatty acid, and a dicarboxylic acid.

In this search, our major efforts were placed on the water extracts of the lunar fines, as somewhat disparate results have been reported on examination of water extracts of the Apollo 11 samples[1–3]. After aqueous reflux of the samples, the water extracts were divided, then analyzed without hydrolysis with 6 *N* HCl. Analyses were also conducted on 1 *N* HCl extracts of the lunar samples, which was the subject of an earlier report[3,8].

The aqueous extracts of Apollo 12 lunar fines have also been investigated for amino acids by classical ion-exchange techniques. NAGY *et al.*[9] reported that on analysis of three samples of lunar fines, the distribution patterns of the trace components were indicative of minor terrestrial contamination, and that *free* amino acids were not present above background levels. Studies conducted by HARADA *et al.*[10] on hydrolyzed aqueous extracts of one trench and five surface samples have shown glycine to be the principal amino acid component. Although these authors did not exclude the possibility of contamination, they reported the amino acid distribution pattern was atypical of terrestrial contamination. They did not find *free* amino acids in Apollo 12 water reflux extracts, however, on acid hydrolysis, the *total amount* for six amino acids was at the 40 to 90 p.p.b. level.

In a manuscript recently published, ZUMWALT *et al.*[6] reported the development of an instrumental-chromatographic device which has greatly simplified the analysis of nanogram quantities of amino acid derivatives by GLC. An injection port-solvent venting system permits the injection of the total derivatized sample (up to 100 $\mu$l) on a standard packed analytical column. This device prevents the large volume of solvent and acylating reagent from traversing the column and disturbing the detection system, thus greatly reducing solvent "tailing". In addition, this system eliminates the TFA interference peak observed on chromatography with ethylene glycol adipate (EGA) columns.

This paper presents the results of our investigations for amino acids in Apollo 11 and 12 lunar fines, and presents a discussion of the capabilities and limitations of analyzing for extremely small amounts of amino acids and other organics by GLC.

## EXPERIMENTAL

### (*I*) *Apparatus and reagents*

The gas chromatographs used in this study included two Hewlett-Packard Model 5750 instruments, each equipped with dual column oven baths, flame ionization detectors, linear temperature programmers, and Honeywell Electronik 16 strip chart recorders.

All amino acids used in this investigation were obtained from Mann Research Laboratories, Inc., New York, N.Y., or Nutritional Biochemicals Corp., Cleveland, Ohio; and were chromatographically pure. Standard amino acid N-trifluoroacetyl *n*-butyl esters were obtained from Regis Chemical Co., Chicago, Ill. The methylene chloride was obtained from Matheson, Coleman and Bell, and the *n*-butanol was a "Baker Analyzed" reagent. The trifluoroacetic anhydride was obtained from Eastman Kodak Co. The micro reaction vials are made by Analytical BioChemistry Laboratories, Inc., Columbia, Mo.

The *n*-butanol and dichloromethane were first refluxed over calcium chloride, then distilled from an all-glass system and protected from atmospheric moisture by storage in glass bottles with inverted ground-glass tops. The HCl gas for preparation of the *n*-butanol·3 *N* HCl was produced by the slow addition of concentrated hydrochloric acid to sulfuric acid, and the evolved HCl gas was passed through two sulfuric acid drying towers before bubbling into the *n*-butanol.

Triply distilled water was obtained with the use of a Kontes Model WS-2 continuous water still.

All glassware was carefully washed in detergent (Alconox) and hot water, rinsed and placed in a bath of sulfuric acid and sodium dichromate at 90–100° for at least 1 h. Each piece of glassware was copiously rinsed with singly distilled water and finally with triply distilled water and dried in an oven at 150°. The glassware was stored in covered stainless steel trays. Finally, each piece of glassware was rinsed with the solvent or reagent used in the analysis.

*(II) Chromatographic columns*

*(A) Column I: ethylene glycol adipate on Chromosorb W*, 0.65 w/w %

(1) *Materials.* Column packing I can be procured from Analytical BioChemistry Laboratories, Box 1097, Columbia, Mo. or Regis Chemical Co., 1101 N. Franklin, Chicago, Ill. EGA, stabilized grade, was obtained from Analabs, Inc., Hamden, Conn. The support material, 80/100 mesh acid washed (AW) Chromosorb W, was obtained from Supelco, Inc., Bellefonte, Pa. Acetonitrile, "Nanograde", was obtained from Mallinckrodt Chemical Works, St. Louis, Mo.

(2) *Procedure.* For preparation of 25 g of column packing I, 24.84 g of AW Chromosorb W were weighed into a 500 ml ridged round bottom flask, then anhydrous "Nanograde" acetonitrile was added until the liquid level was *ca.* 1/8 in. above the Chromosorb. 10 ml of a solution containing 16.25 mg/ml of stabilized EGA in anhydrous "Nanograde" acetonitrile were then added to the flask containing the Chromosorb W. The flask was then placed on a rotary evaporator, *slowly* removing the solvent at room temperature under a partial vacuum for *ca.* 45 min. When the Chromosorb was still slightly damp, the vacuum was increased and the flask immersed in a 60° water bath with continued rotation until the solvent was completely removed. At this point, no Chromosorb W packing should adhere to the inner wall of the flask during rotation. At the end of this period, the dry, freely-flowing column packing was poured into clean, dry 1.5 m × 4 mm I.D. glass columns with gentle tapping. Dry silanized glass wool plugs were then placed in each end of the column to hold the packing in place. Prior to analytical use, the column was placed in the gas chromatograph and conditioned at 220° with a carrier flow of *ca.* 50 ml/min of pure $N_2$. Analyses could be made after conditioning for 1 h when 0.5–1 $\mu$g of each amino acid were injected, however, longer conditioning times (8–24 h at 220°) were required for analyses at the nanogram level.

*(B) Column II: OV-17 on H.P. Chromosorb G, 1 w/w %*

(1) *Materials.* OV-17 was obtained from Supelco, Inc. Chromosorb G, H.P., 80/100 mesh was purchased from Applied Science Labs., State College, Pa. Dichloromethane, "Nanograde" was procured from Mallinckrodt Chemical Works. A solution was prepared containing 10 mg/ml of OV-17 in dichloromethane.

(2) *Procedure.* For preparation of 30 g of column packing II, 29.7 g of H.P. Chromosorb G were weighed into a 500 ml ridged round bottom flask, then "Nanograde" dichloromethane was added until the liquid level was *ca.* 1/8 in. above the support material. 30 ml of the OV-17 solution (300 mg) were pipetted into the flask. The solvent was removed in the manner described above for preparation of column packing I, and the dried column packing placed in 1.5 m × 4 mm I.D. glass columns. The columns were then placed in the gas chromatograph, and conditioned overnight at 250° with a carrier gas flow of *ca.* 50 ml/min of pure $N_2$. A thorough discussion of chromatographic columns for analysis of extremely small amounts of amino acids is presented in refs. 6 and 7 and includes conditioning, experimental, and storage of the column. The solvent vent-chromatographic device is available from Analytical BioChemistry Laboratories, Inc., Box 1097, Columbia, Mo.

*(III) Extraction of lunar fines*

*(A) Sand blank*

The sand blank was prepared by heating a sample of Ottawa sand (Matheson, Colman and Bell) for 48 h at 1000°.

*(B) Apollo 12*

A 1.2 g sample of the Apollo 12 lunar fines (ARC 12023.04) was placed in a 10 ml round bottom flask. 6 ml of triply distilled water were added, a reflux condenser was attached, and the mixture was brought to a gentle boil, then refluxed for 17 h.

At the end of this time, the flask was allowed to cool and the water extract was decanted into a 15 ml conical pyrex glass centrifuge tube. The tube was closed with a teflon lined screw cap, then centrifuged at 2700 r.p.m. for 5 min to remove fines suspended in the liquid. A slight turbidity of very fine material remained in suspension.

The water extract was again decanted into a 10 ml beaker and reduced in volume to *ca.* 1 ml by mild heating under an infrared lamp. The solution was then transferred to a micro reaction vial for derivatization to the N-TFA *n*-butyl esters.

*(C) Basalt*

A 1 g sample of a basalt bomb obtained from near Aloi Crater, Island of Hawaii (NASA 042070.2) was likewise refluxed with 6 ml of triply distilled water for 17 h, and the aqueous extract was taken through the entire analytical chromatographic procedure with the Apollo 12 extract to obtain a blank for the analytical method.

*(D) Apollo 11*

A 2.2 g sample of Apollo 11 lunar fines (10086-3 bulk A) was placed in a 50 ml pear-shaped flask, 10 ml of triply distilled water were added, and the mixture was refluxed for 17 h as described. The decanted water extract was centrifuged at 2700 r.p.m. for 5 min, then divided into two equal parts; one part was derivatized and analyzed directly by GLC, and the other part was subjected to hydrolysis with 6 *N* HCl followed by derivatization and GLC analysis.

(*E*) *Hydrolysis of water extract*

The water extract obtained from the Apollo 11 sample was evaporated to near dryness in a 10 ml beaker under an infrared lamp, then transferred with 2 ml of 6 *N* HCl into an 8 ml pyrex glass hydrolysis tube with a teflon lined cap. The sample extract was then heated in an oven for 4 h at 145° according to the procedure described by ROACH AND GEHRKE[11]. At the end of this period, the tube was removed from the oven, allowed to cool, then transferred to a micro reaction vial for derivatization and GLC analysis.

(*F*) *Reagent and performance studies*

Samples were also prepared to evaluate the purity of the reagents used in each step of the analytical procedure. The water, 6 *N* HCl, *n*-butanol·3 *N* HCl, trifluoroacetic anhydride, and dichloromethane were taken through the entire chemical and analytical procedures singly and in combinations as companion blanks for the Apollo 11 and 12 analyses. Concurrently, performance standards were prepared and analyzed to confirm the integrity of the derivatization reagents and chromatographic system.

(*G*) *Derivatization of Apollo* 11 *and* 12 *water extracts*

(1) For the conversion of amino acids to the N-TFA *n*-butyl esters, the aqueous solutions were placed in micro reaction vials, and evaporated just to dryness under an infrared lamp. Samples of larger volume were placed in 5–10 ml beakers, evaporated to *ca.* 0.5 ml, then transferred to the vial with triply distilled water. A black surface was used to support the samples, thus increasing the efficiency of the evaporative step. After all visible moisture was removed from the reaction vials, the vials were tightly closed with the teflon lined caps and allowed to cool. Then 50 $\mu$l of anhydrous dichloromethane were added, and evaporated under the infrared lamp to azeotropically remove the last traces of moisture.

(2) For esterification of the amino acids to the *n*-butyl esters, 100 $\mu$l of *n*-butanol·3 *N* HCl were added to the reaction vials. The vials were then tightly capped and placed in the ultrasonic bath just to the liquid level in the vial for *ca.* 30 sec. Water from the ultrasonic bath must be carefully excluded in this step. The exterior of the vials were dried with tissue.

(3) The vials were then placed in a 100° sand bath for 30 min. Only the lower portion of the vial containing the liquid was submerged in the sand, thus maintaining a liquid phase and allowing the sample to reflux. The samples were then removed from the sand bath and allowed to cool.

(4) After the esterification step, the samples were evaporated *just to dryness* by removing the cap from the vials, and placing them under the infrared lamp.

(5) The vials were then tightly capped, allowed to cool, then 50 $\mu$l of dichloromethane were added and evaporated under the infrared lamp as in step (1).

(6) The amino acid *n*-butyl esters were trifluoroacylated by the addition of 100 $\mu$l of TFAA–$CH_2Cl_2$ solution. The amount of TFAA present was varied from 0.5 to 10 $\mu$l per 100 $\mu$l of solution. The vials were again tightly capped, sonicated for *ca.* 30 sec, then heated in the 100° sand bath for 10–20 min, depending on the wall thickness of the reaction vial used. For the analysis of arginine, tryptophan, and cystine, acylation at 150° for 5–10 min yields more quantitative results. After allowing the samples to cool to room temperature, analyses were made by GLC.

*(IV) Comments on the derivatization method*

(A) The purity of all reagents and the cleanliness of all glassware used throughout the procedure is of obvious importance when analyzing at these low levels of amino acid concentration. The distillation of the required reagents, described in section I, must be carefully conducted. To monitor the purity of the redistilled *n*-butanol and dichloromethane, 10-ml aliquots of the redistilled products were evaporated to *ca.* 0.25 ml, then analyzed by GLC under the chromatographic conditions used for analysis of the derivatized sample. The Drierite or molecular sieve over which the distilled solvents are stored was rinsed with portions of the anhydrous reagent prior to use. All reagent bottles must be kept tightly closed and covered to exclude atmospheric moisture and air-borne dust particles.

(B) Effective techniques for obtaining clean glassware for use in the derivatization procedure include soaking the glassware in hot chromic acid cleaning solution for 4–8 h, followed by exhaustive rinsing with triply distilled water. Alternatively, the necessary glassware may be placed in a furnace and heated overnight at 500° to remove contaminating organics, and final rinsing with the reagents to be used.

(C) Moisture must be carefully excluded during the entire derivatization procedure. The *n*-butanol, HCl gas, and dichloromethane must be anhydrous, and the TFAA should not contain significant amounts of TFA.

(D) It is essential that reagent blanks be analyzed particularly often, as amino acid contamination can be easily introduced from a variety of sources, especially from fingerprints.

(E) Performance standards must be analyzed to ensure the integrity of the derivatization reagents. The concentration of the amino acids in the performance standard should be similar to the amino acid concentrations in the samples to be analyzed.

*(V) GLC instrument settings and conditions*

The chromatograms presented were obtained from a strip chart recorder with 1 mV full scale response. The amplification of the signal from the hydrogen flame detector was accomplished by use of an electrometer with a maximum sensitivity of $1 \times 10^{-12}$ A/mV. The designation $\times 1$ appearing on the figures denotes this sensitivity as maximum. Other sensitivity designations (*e.g.* $\times 2$, $\times 2.5$, etc.) represent changes in sensitivity by that particular factor. The changes in sensitivity during the analyses were made to obtain the optimum amount of information, although they do not add to the convenience of interpretation. The symbols ADJ appearing on the chromatograms signify changes in the suppression amperage only and do not alter the sensitivity setting of the analysis.

## RESULTS AND DISCUSSION

Initial studies included the analysis of blanks of the materials and reagents used for the GLC analysis of amino acids. A chromatographic column blank is presented in Fig. 1, with no sample injected. The extraneous peaks that were eluted above 200° did not significantly interfere with the analysis for amino acids, as only the more complex protein amino acids were eluted above this temperature, *i.e.* tyrosine, glutamic acid, lysine, arginine, tryptophan, and cystine. Interest was primarily

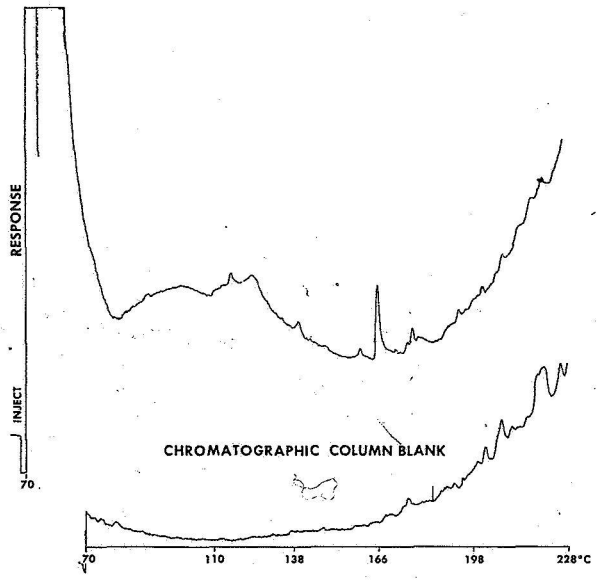


Fig. 1. Chromatographic column and reagent blank. 100 $\mu$l *n*-butanol·3 *N* HCl and 100 $\mu$l TFAA–$CH_2Cl_2$ (0.5 $\mu$l/200 $\mu$l). Final volume, 50 $\mu$l; injected, 5 $\mu$l; initial temperature, 70°; initial hold, 6 min; program rate, 4°/min; final temperature, 228°; attenuation, $4 \times 10^{-12}$ AFS; injection port, 280°. Column, 1.0 w/w % OV-17 on 80/100 mesh H.P. Chromosorb G, 2.5 m × 2 mm I.D. glass.

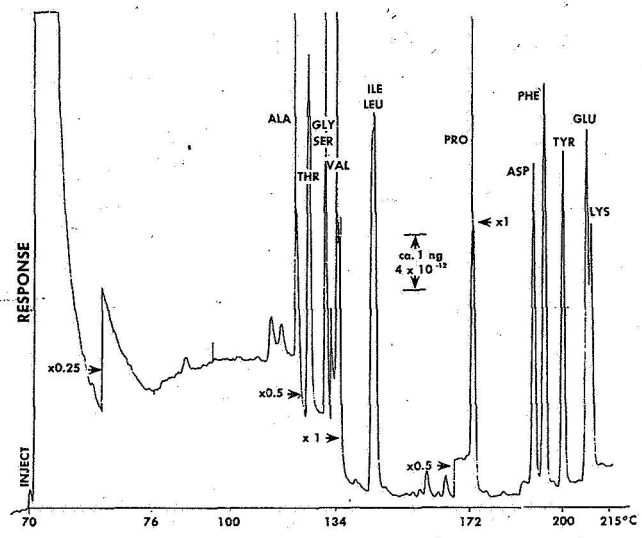


Fig. 2. Performance-chromatography standard. Derivatization and GLC analysis of standard amino acid mixture. Derivatized, 1 $\mu$g of each; injected, 25 ng of each; initial temperature, 70°; initial hold, 6 min; program rate, 4°/min; final temperature, 215°; attenuation, $16 \times 10^{-12}$ AFS; injection port, 280°. Column, 1.0 w/w % OV-17 on 80/100 mesh H.P. Chromosorb G, 2.5 m × 2 mm I.D. glass.

centered on alanine, glycine, threonine, serine, and aspartic acid, as small amounts of these and other less complex compounds were reported in samples of Apollo 11 lunar fines by other investigators[1,2]. Also, Fig. 1 presents the reagent and chromatographic blank used for derivatization and analysis of amino acids as their N-trifluoroacetyl *n*-butyl esters. The small peak eluted at 166° did not correspond in retention temperature to any of the amino acid derivatives.

A derivatization performance study was then conducted to confirm that the employed derivatization reagents were in sufficient molar excess to quantitatively derivatize the amino acids. This information was of particular importance as the amount of TFAA used to acylate the samples was deliberately kept small, in order to suppress the TFA interference peak when chromatographing on the ethylene glycol adipate column for confirmation of any amino acids which might be found. Therefore, a relatively large amount (1 $\mu$g) of each amino acid was used to evaluate the acylating

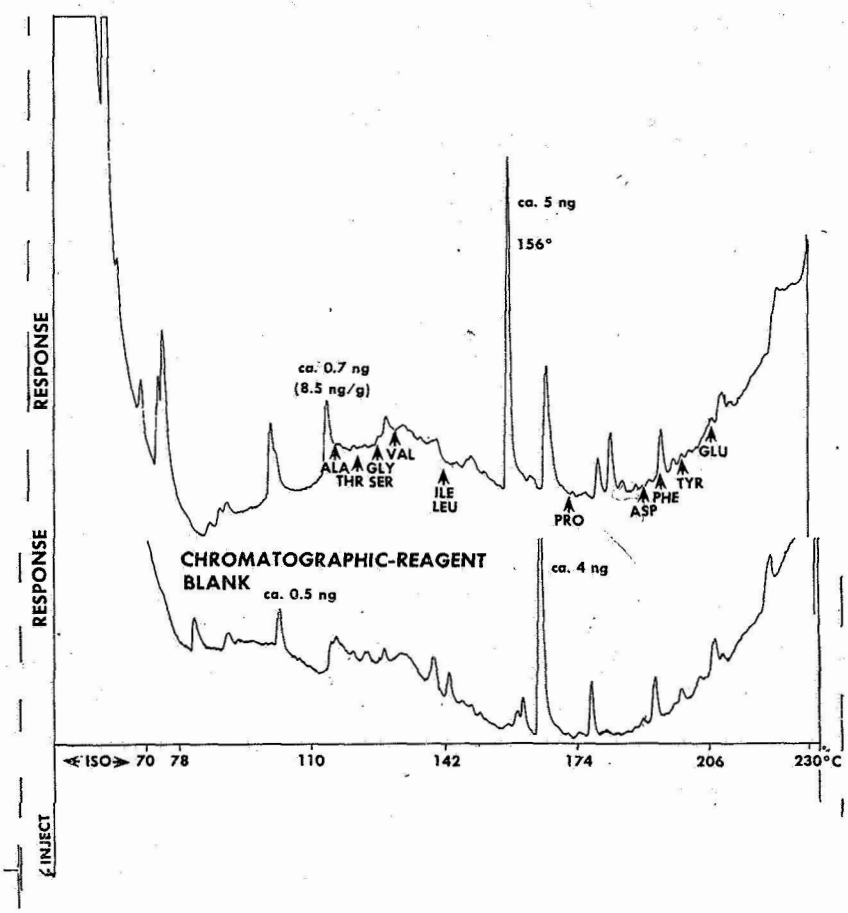


Fig. 3. Apollo 11 water extract; 3 × distilled water, reflux 17 h. Sample, 1.1 g of lunar fines (3.3 ml). 100 $\mu$l *n*-butanol·3 *N* HCl and 100 $\mu$l TFAA–$CH_2Cl_2$ (0.5 $\mu$l/100 $\mu$l). Final volume, 60 $\mu$l; injected, 5 $\mu$l; initial temperature, 70°; initial hold, 6 min; program rate, 4°/min; final temperature, 232°; attenuation, 4 × $10^{-12}$ AFS; injection port, 280°. Column, 1.0 w/w% OV-17 on 80/100 mesh H.P. Chromosorb G, 2.5 m × 2 mm I.D. glass.

reaction of the TFAA–dichloromethane solution. The derivatization reagents were found to be suitable, yielding large peaks for 25 ng of each amino acid, as seen in Fig. 2. Several attenuation changes were made during this analysis to obtain information on the instrumental sensitivity. 1 ng gave *ca.* a 10 % full scale response at an attenuation setting of $4 \times 10^{-12}$ AFS. The derivatization was found to be essentially quantitative, as seen by dilution and analysis of standard amino acid mixtures.

The water extract of the Apollo 11 sample was then examined. The extract was divided equally, one part derivatized and analyzed directly, and the remaining part hydrolyzed with 6 *N* HCl at 145° for 5 h then taken through the derivatization and GLC procedures. Concurrently, complete reagent blanks were prepared as parallel samples, and were analyzed under the same experimental and chromatographic conditions as the derivatized lunar extracts.

Fig. 3 presents the chromatograms obtained on analysis of the water extract

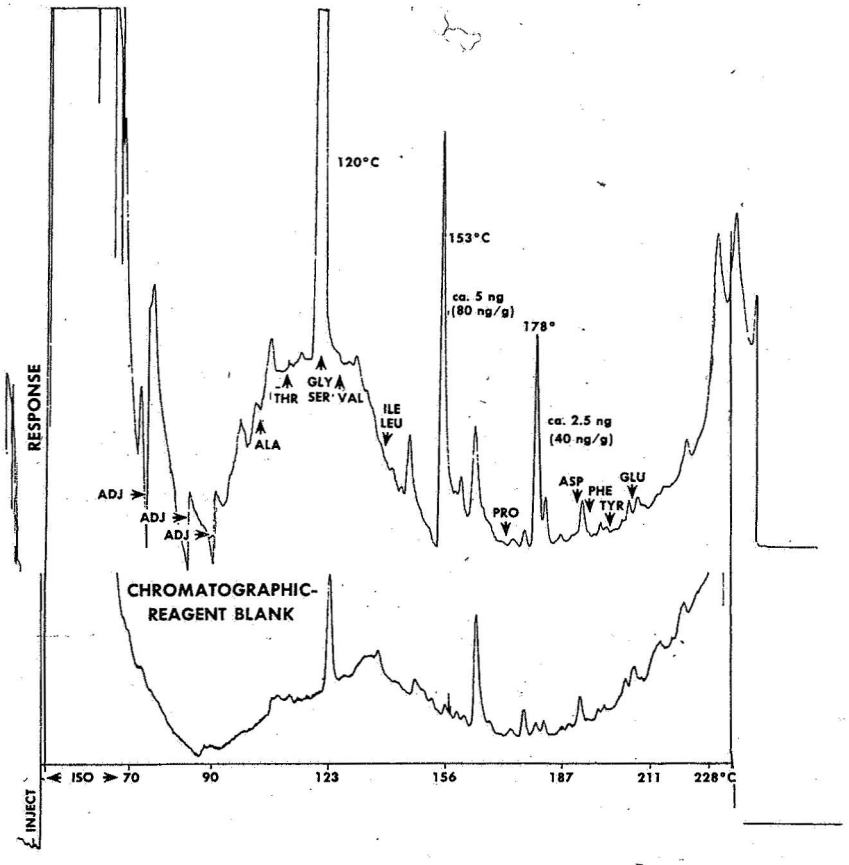


Fig. 4. Apollo 11 water extract, hydrolyzed; 3 × distilled water, reflux 17 h. Sample, 1.1 g of lunar fines (3.3 ml), hydrolyzed with 6 *N* HCl, 5 h at 145°. 100 μl *n*-butanol·3 *N* HCl and 100 μl TFAA–$CH_2Cl_2$ (0.5 μl/100 μl). Final volume, 80 μl; injected, 5 μl; initial temperature, 70°; initial hold, 6 min; program rate, 4°/min; final temperature, 232°; attenuation, $4 \times 10^{-12}$ AFS; injection port, 280°. Column, 1.0 w/w % OV-17 on 80/100 mesh H.P. Chromosorb G, 2.5 m × 2 mm I.D. glass.

corresponding to 1.1 g of the Apollo 11 sample, and the companion chromatographic-reagent blank. These analyses show that less than 4 ng of each amino acid were present per g of lunar material.

The analysis of the hydrolyzed water extract, also corresponding to 1.1 g of Apollo 11 fines, resulted in the chromatogram presented in Fig. 4, along with the companion chromatographic-reagent blank. Again, no amino acids were found above the 4 ng/g level. The large peak eluted at 120° occurred in the glycine–serine region of the chromatogram, therefore no conclusions could be drawn regarding these amino acids. A smaller peak eluted at the same temperature was observed in the corresponding reagent blank. The peaks that were observed at 153° and 178° exhibited retention times distinctly different from the amino acid derivatives, and could not be characterized.

The chromatogram obtained on analysis of the derivatized water extract of 1.2 g of Apollo 12 lunar samples is shown in Fig. 5. Concurrently, an equivalent amount of basalt was extracted with water under the same conditions (13 h under reflux), then derivatized and analyzed to serve as a complete reagent and procedural blank of the method. The Apollo 12 water extract gave no indication of containing indige-

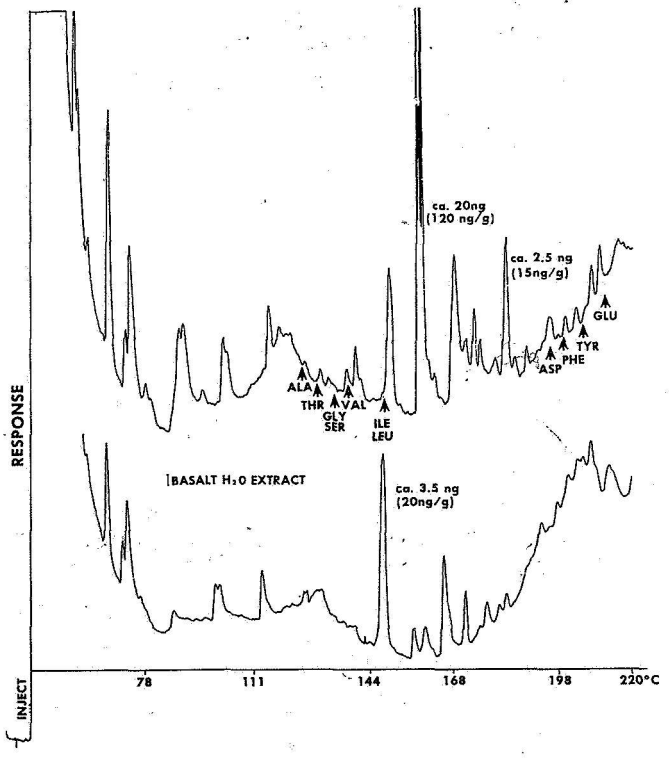


Fig. 5. Apollo 12 water extract; 3 × distilled water, reflux 13 h. Sample, 1.2 g of lunar fines (6 ml). 100 μl of *n*-butanol·3 *N* HCl and 100 μl TFAA–$CH_2Cl_2$ (0.5 μl/100 μl). Final volume, 30 μl; injected, 5 μl; initial temperature, 70°; initial hold, 6 min; program rate, 4°/min; final temperature, 220°; attenuation, 4 × $10^{-12}$ AFS; injection port, 280°. Column, 1.0 w/w % OV-17 on 80/100 mesh H.P. Chromosorb G, 2.5 m × 2 mm I.D. glass.

nous amino acids at a level greater than 5 ng/g *each* in the lunar soil. The large peak eluted at 156° (corresponding to *ca.* 120 ng/g) was not identified.

Analyses of the water extracts of the returned lunar samples (Apollo 11 and 12) resulted in none of the common amino acids being detected at concentration levels (as *free* amino acids) of *ca.* 4–5 ng/g. However, several unidentified chromatographic peaks were observed, particularly in the Apollo 12 sample, which were not present in the corresponding procedural blanks. These substances which appear to be derivatizable, could either be indigenous to the sample, contamination, or the results of the unusual chromatographic effect reported earlier by these authors[8].

At the 2nd Lunar Science Conference, Houston, January, 1971, Fox and coworkers[10] reported that they did not find *free* amino acids in Apollo 12 fines at the nanogram level, however, they did find amino acids after 6 *N* HCl hydrolysis of the aqueous extract. NAGY *et al.*[9] reported at this conference that *free* amino acids in Apollo 12 were not present at a level greater than background. A major peak at 120° was observed in the Apollo 11 *hydrolyzed* water extract. Two other major peaks were also observed at 153 and 178°. These may be due to the observation reported by FOX. Further comfirmation is needed.

To evaluate other classes of organic compounds in addition to amino acids that could be detected by the derivatization and chromatographic methods used, a synthetic solution containing an alcohol, an amine, a fatty acid, a dicarboxylic acid, and an amino acid was studied. Fig. 6 presents the chromatogram obtained after derivatization and chromatography of the standard, indicating that these classes of compounds were amenable to detection by the analytical method used.

Of interest in this search was a knowledge of the amount of amino acids which

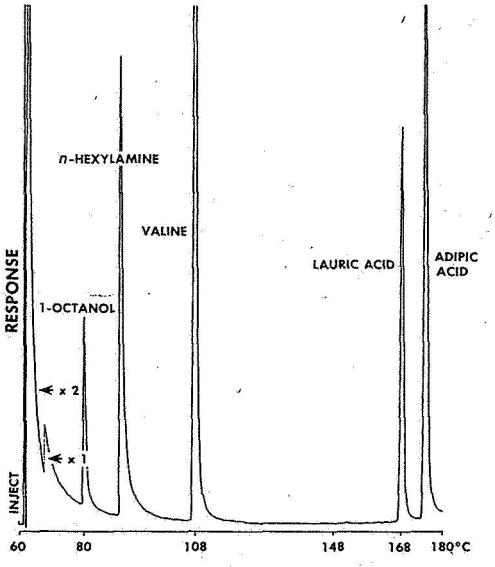


Fig. 6. Performance and chromatographic standard. Derivatization and GLC analysis of a five-component mixture. Sample injected, *ca.* 1 μg of each; attenuation, $2 \times 10^{-10}$ AFS = × 1; initial temperature, 60°, program rate, 6°/min; final temperature, 180°; injection port, 280°. Column, 3.0 w/w % OV-17 on 80/100 mesh H.P. Chromosorb W, 1.5 m × 4 mm I.D. glass.

might be expected to originate on contamination by a *clean single* fingerprint. The GLC analysis of fingerprint amino acids was presented in an earlier manuscript[6], and showed that a minimum of *ca.* 1 $\mu$g of *total* amino acids could be expected per "clean" fingerprint (Fig. 7). HAMILTON[12] had earlier investigated contamination from amino acids resulting from thumbprints by classical ion-exchange chromatography and reported that the detection of each of seventeen amino acids ranged from *ca.* 500 to 20 ng per dry thumbprint. He reported that serine and glycine were the most abundant amino acids resulting from wet thumbprints with *ca.* 10000 and 7000 ng, respectively.

*Injection port solvent vent-chromatographic system*

Analyses of samples containing submicrogram quantities of seventeen protein amino acids were successfully conducted, using the derivatization procedure described in the EXPERIMENTAL section and the injection port solvent vent-chromatographic system. Fig. 8 shows the chromatogram obtained after an aqueous solution containing 5 ng of *each* amino acid was taken through the *total* derivatization and GLC chromatographic method. The derivatization and instrumental conditions are given in the figure legend.

## CONCLUSIONS

The examination of unhydrolyzed water reflux extracts of Apollo 11 and 12 lunar fines by a highly sensitive gas–liquid chromatographic method resulted in none of the common amino acids being identified at a concentration of *ca.* 3 to 5 ng/g of each. These observations are in agreement with the results reported by NAGY AND

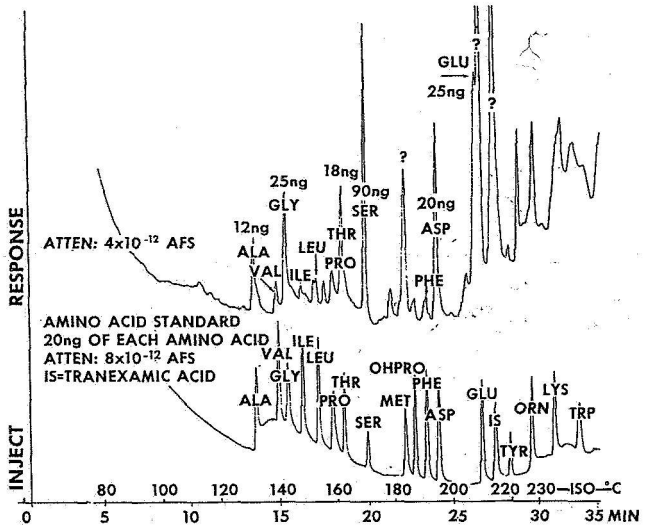


Fig. 7. GLC analysis of a fingerprint. Amino acid N-TFA *n*-butyl esters. Final sample volume, 60 $\mu$l; injected, 45 $\mu$l; vent time, 45 sec; initial temperature, 55°; program rate, 6°/min; final temperature, 230°. Column, 0.65 w/w % EGA on 80/100 mesh Chromosorb W, with solvent venting system.

HAMILTON[9] and HARADA *et al.*[10], using classical ion-exchange methods in that *free* amino acids were not found above background levels.

At this date, the only divergence of results obtained by the various investigators is the presence or absence of amino acid precursors in water reflux extracts of lunar fines, which yield some amino acids on hydrolysis with 6 *N* HCl. Fox and co-workers[10] have reported the presence of amino acids precursors in the Apollo 12 fines which gave rise on hydrolysis to a *total* of 20 to 90 ng/g of amino acids. NAGY AND HAMILTON did not report results on hydrolysates of the aqueous extracts. Although we did not confirm the findings of Fox, several prominent non-amino acid peaks were found in the chromatograms obtained *after hydrolysis* of the water extract of an Apollo 11 sample. In particular, a large unidentified peak occurred in the glycine–serine region (*ca.* 120 ng/g) and two other peaks were observed at 153° (80 ng/g) and 178° (40 ng/g). An acidic hydrolysate of the aqueous extract of Apollo 12 fines was not made. Further confirmation experiments will be necessary byGLC, GLC–MS, and classical ion-exchange (CIE) for an unequivocal conclusion as to the formation of amino acids on hydrolysis of precursors.

Based on the reports of Apollo 11 and 12 investigations, the unequivocal de-

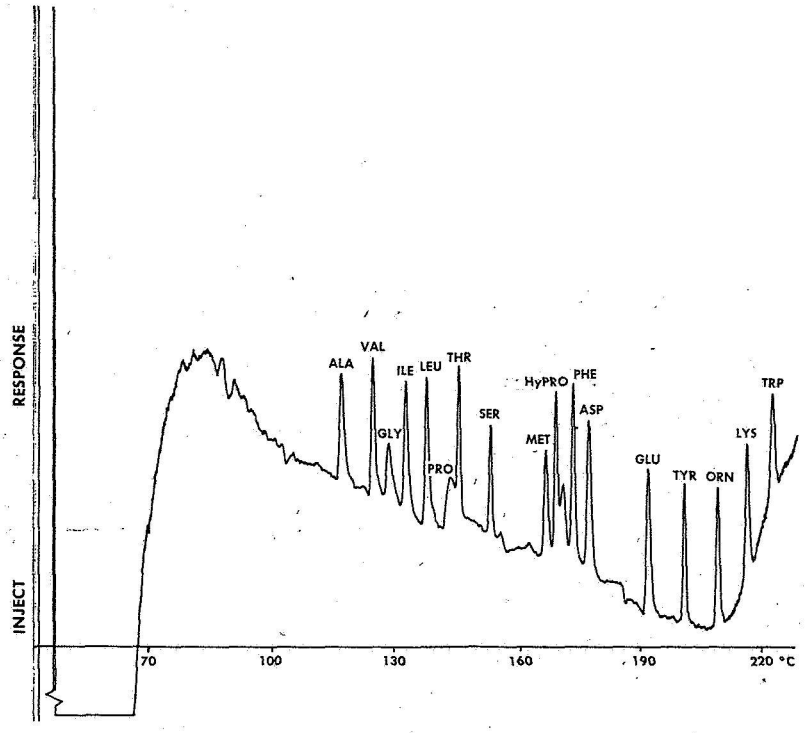


Fig. 8. Derivatization and GLC analysis of 5 ng of each amino acid. Derivatization: esterification, 25 $\mu$l *n*-butanol·3 *N* HCl at 100° for 70 min; acylation, 25 $\mu$l $CH_2Cl_2$–TFAA (9:1) at 100° for 20 min. GLC analysis: mode, dual differential; injection port solvent vent. Sample injected, 25 $\mu$l; solvent vent time, 30 sec; injection port temperature, 150°; initial temperature, 70°; initial hold, 4 min; program rate, 6°/min; attenuation, 8 × $10^{-12}$ AFS. Column, 0.65 w/w% EGA on 80/100 mesh AW Chromosorb W, 1.5 m × 4 mm I.D. glass. Precolumn, 1.0 w/w% OV-17 on 80/100 mesh H.P. Chromosorb G, 4 in. × 4 mm.

termination of the existence of indigenous amino acid precursors in lunar samples is a formidable task, as the concentration of these compounds, if present, is extremely small. Therefore, with this objective in mind, a series of interlaboratory experiments has been designed by the participating investigators, PONNAMPERUMA, KVENVOLDEN, GEHRKE; FOX, HARE, HARADA; NAGY, HAMILTON; ORO; and FLORY) to thoroughly evaluate and optimize the GLC–MS and CIE analytical methods used to detect and elucidate the structure of these compounds. This study will be conducted in advance of, and will be aimed at, obtaining unequivocal results from Apollo 14 analysis. These experiments will include: (a) an assessment of sensitivity of methods on a series of standard amino acid solutions at the 5, 20, and 100 ng levels by each group, using both CIE and GLC methods, and, (b) analyses for amino acids in hydrolyzed and unhydrolyzed water extracts of a single homogeneous sample of Onverwacht cherts to determine presence of precursors. These studies will aid in determining the most useful techniques for extraction, hydrolysis, and detection of the amino acids in samples of Apollo 14.

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